Evaluation of Unstructured Kinetic Models for the Production of Bioethanol from Banana and Pineapple Wastes

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Bioethanol is a renewable energy source, and its production from agricultural wastes, such as banana and pineapple peels, is an economical approach. Enzymatic hydrolysis experiments were performed using a simultaneous saccharification and co-fermentation (SSCF) method. Banana and pineapple wastes inoculated with *Aspergillus terreus* and *Kluyveromyces marxianus* produced the maximum ethanol concentrations of 0.35 g/L and 0.27 g/L, respectively. Furthermore, logistic unstructured and incorporated models described well the growth of microorganism, product formation, and substrate utilization during SSCF system with high R^2 and low RMSD.

Keywords: Banana waste; Pineapple waste; Bioethanol; Simultaneous saccharification and co-fermentation (SSCF); Unstructured models; Kinetic

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INTRODUCTION

It is well known that fossil fuels are non-renewable resources and most of the world's energy comes from fossil fuels (Dresselhaus and Thomas 2001), but due to their eventual exhaustion, alternative fuels have been developed. One such alternative, ethanol, can be produced from ethylene, a petroleum by-product, or fermentation (Taherzadeh and Karimi 2008). Synthetic ethanol is produced by the hydration of ethylene. However, ethylene is also a non-renewable resource, and the process requires a high temperature and pressure and, consequently, a lot of energy. In contrast, bioethanol can be produced from plant materials by fermentation. The most important aspect of this method is the availability of plant materials. Generally, the juice is extracted from sugar-based feedstock, which is then fermented and distilled into 90% to 95% ethanol (Ghosh and Nag 2008). According to Balat and Balat (2009), all bioethanol in Brazil is produced from sugar cane.

All materials that can be converted into sugar, such as cellulosic materials, are potential sources of bioethanol. The bioethanol from cellulosic materials requires a few more processing steps than traditional bioethanol produced from sugar cane. Cellulosic materials must be hydrolyzed to break down fermentable sugars prior to reducing the sugars to ethanol (Sun and Cheng 2002). The efficiency of this process is affected by several factors, *i.e.* porosity, cellulose fiber crystallinity, and lignin and hemicellulose contents (McMillan 1994; Sun and Cheng 2002). Therefore, this bioethanol production method is more expensive, which is a major bottleneck for commercial applications.

In addition, a sudden fall in the price of gasoline inevitably gives the impact to the margins of ethanol production owing to the recessionary pressures. However, according to the EIA (2016), the U.S Environmental Protection Agency (EPA) finalized a rule setting Renewable Fuel Standard (RFS) volumes to develop the current Short-Term Energy Outlook forecast. According to the report, ethanol production averaged an estimated 965,000 barrels per day in 2015, and it is forecast to average close to that level in both 2016 and 2017. On the other hand, ethanol consumption averaged 910,000 barrels per day in 2015, and it is forecast to average more than 920,000 barrels per day in both 2016 and 2017. The demand for bioethanol is still strong according to the EIA (2016).

In order to see the continued profits to the ethanol producers, efforts have been made to improve the efficiency of the cellulosic bioethanol production and, thus, reduce the cost of its production. One way to reduce costs is to utilize waste materials with high cellulose content. Since such wastes have little or no other value, researchers or ethanol producers are motivated to utilize the agriculture waste instead of corn or sugar cane.

Agriculture is an important sector that contributes to the socio-economic growth of Malaysia, providing employment for more than 10% of the population. In addition to rubber, palm oil, and cocoa, Malaysian farmers produce a variety of fruits including bananas, pineapples, rambutan, *etc.* Banana production recorded best volume growth in year 2012 with 336,000 tonnes, whereas pineapples recorded 334,000 tonnes in Malaysia. Both banana and pineapples are listed in Malaysia's top ten crops (Agriculture and Agri-Food Canada 2014). Thus, there is abundantly available agricultural biomass that could be used for bioethanol production in Malaysia. A secondary benefit to bioethanol production from agricultural biomass is the reduction of environmental pollution caused by fruit waste residues. Large amounts of agricultural biomass will be disposed in several ways including for landfill or in the river (causing effluent) and consequently requires the waste water treatment. This is not economical. So, the fascinating solution is to utilize the fruit waste residues to produce the bioethanol.

Though a banana peel is a fruit residue, it accounts for 30% to 40% of the total fruit weight and contains large amounts of carbohydrates, proteins, and fiber but low quantities of lignin (Hammond *et al.* 1996). Furthermore, the waste derived from pineapple (*Ananas cosmosus*) contains 25.8% reducing sugars and 11.2% cellulose (Rani and Nand 2004). Castro *et al.* (2011) also reported that pineapple peel juice contains 2.14% w/v glucose, 2.4% w/v fructose, and 2.10% w/v sucrose. Simultaneous saccharification and fermentation (SSF) or simultaneous saccharification and co-fermentation (SSCF) methods both perform well in the bioconversion of lignocellulosic materials to bioethanol. According to the Kang *et al*. (2011), SSCF results in 3.25 wt% ethanol yield; there is 2.65 wt% ethanol yield *via* SSF using paper sludge as feed. Koppram *et al*. (2013) also reported that operating SSCF at high insoluble solids not only assists mixing but also yields high ethanol production.

This study focuses on banana and pineapple wastes as potential substrates for bioethanol production. Kinetic analysis was necessary to understand the rates of cell synthesis, product formation, and substrate consumption. Hence, the kinetics of bioethanol production was studied using the Logistic model and the Luedeking-Piret model *via* Levenberg-Marquardt (L-M) method.

EXPERIMENTAL

Preparation of Substrates

Banana and pineapple peel wastes were obtained from UniMAP Kampus UniCITI Alam, Perlis, Malaysia. The substrates were washed and dried in oven at 50 °C and milled into 0.5-mm particles.

Microorganisms

Aspergillus terreus (fungus) and *Kluyveromyces marxianus* (thermo-tolerant yeast) were obtained from the School of Bioprocess Engineering, UniMAP, Arau, Malaysia. *A. terreus* and *K. marxianus* were grown on malt extract agar and potato dextrose agar, respectively, and incubated at 30 °C for 5 days.

Preparation of Mycelia Suspension

The mycelia suspension was prepared by suspending mycelia discs from 7-day-old culture plates in sampling bottles containing sterilized distilled water and 0.1% (v/v) polysorbate 80 (Tween® 80). A disc of 5 mm in diameter was cut on the mycelia mats of the agar plate using a sterile cork borer. A total of 10 discs were placed into 100 mL of sterilized distilled water and vortexed for 5 min to homogenize the suspensions.

Preparation of Yeast Colonies

For fermentation cultures, a colony from the agar plate was into a culture medium containing 50 g/L glucose, 2.5 g/L yeast extract, 5 g/L peptone, 1 g/L KH_2PO_4, 0.62 g/L $MgSO_4.7H_2O$, and 2.5 g/L $(NH_4)_2SO_4$. The culture was grown at 38 °C.

Enzymatic Hydrolysis

Enzymatic hydrolysis experiments were performed using simultaneous saccharification and co-fermentation (SSCF). An inoculum of 10% (v/v) of *A. terreus* and *K. marxianus* (OD_{45} = 0.5) were added to 50 g/L of substrates (100 mL working volume), and the experiment was carried out at 38 °C and pH 5.5 for 5 d in 250-mL Erlenmeyer flasks. During the first 12 h, SSCF was carried out on a rotary incubator with agitation at 150 rpm. The shaker speed was increased to 250 rpm after 12 h. Samples were taken periodically and centrifuged at 4500 rpm for 15 min. The precipitate was used to determine the culture dry weight, while the supernatant liquid was used for the analysis of soluble sugar and ethanol. All experiments were conducted in triplicate.

Selected Unstructured Models of Fermentation Kinetics

The kinetic parameters for each model were obtained from a non-linear least-square regression, in Polymath software (Version 5.1, CACHE Corp., Storrs, CT, USA).

Microbial growth kinetics

The logistic model is a substrate-independent model that describes the inhibition of biomass on mycelia growth during batch fermentation (Teoh 2014). Microbial growth is governed by a hyperbolic relationship (Rajendran and Thangavelu 2008), and in this model, the limit to the maximum attainable biomass concentration is described by Eq. 1,

$$\frac{dX}{dt} = \mu_o X\left(1 - \frac{X}{X_m}\right) \quad (1)$$

where X is the biomass, x_m is the maximum attainable biomass concentration, and μ_o is the initial specific growth rate.

The integrated form of Eq. 1 using $X = X_o$ ($t = 0$) gives a sigmoidal variation of X as a function of t, which represents the exponential and stationary phases (Eq. 2):

$$X(t) = \frac{X_o e^{\mu_o t}}{1-(\frac{X_o}{X_m})(1-e^{\mu_o t})} \quad (2)$$

Product formation kinetics

The kinetics of bioethanol production are based on the Luedeking-Piret equation, in which the product formation rate is linearly correlated with both the instantaneous biomass concentration (X) and the growth rate (dX/dt) (Rajendran and Thangavelu 2008). Equation 3 describes the classical Luedeking-Piret model, which combined the growth and non-growth associated contributions to product formation (Chavez-Parga *et al.* 2008; Teoh 2014),

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (3)$$

where P is the product concentration, and α and β are empirical constants that vary with the fermentation conditions. The product formation (P) was expressed as a function of time (t) and could be written as shown in Eq. 4.

$$dP = \alpha dX + \beta \int X(t)dt \quad (4)$$

In this study, the basic logistic model (Eq. 1) was incorporated into the Luedeking-Piret model (Eq. 4) for $X(t)$, under conditions when P and t are equal to 0. The final product formation rate equation is shown in Eq. 5.

$$P(t) = P_0 + \alpha X_0 \left[\frac{e^{\mu_o t}}{1-\left(\frac{X_o}{X_m}\right)(1-e^{\mu_o t})} - 1\right] + \beta \frac{X_m}{\mu_0} \ln[1 - (\frac{X_o}{X_m})(1 - e^{\mu_o t})] \quad (5)$$

Substrate consumption kinetics

The substrate consumption kinetic was given by Eq. 6, which considers the substrate conversion to biomass, to product, and also the substrate consumption for maintenance (Teoh 2014),

$$\frac{dS}{dt} = \frac{1}{Y_{X/S}} \frac{dX}{dt} - \frac{1}{Y_{P/S}} \frac{dP}{dt} - m_S X \quad (6)$$

where $Y_{X/S}$ is the biomass yield coefficient, $Y_{P/S}$ is the product yield coefficient, and m_S is the maintenance coefficient for microbes. Hence, rearranging Eq. 6 produced the modified Luedeking-Piret equation (Eq. 7),

$$\frac{dS}{dt} = \gamma \frac{dX}{dt} - \delta X \quad (7)$$

where $\gamma = 1/Y_{X/S} + \alpha/Y_{P/S}$ and $\delta = \beta/Y_{P/S} + m_S$.

In general, the substrate consumption (S) was expressed as a function of time and could be written as in Eq. 8.

$$-dP = \gamma dX + \delta \int X(t)dt \quad (8)$$

In this study, the basic logistic model (Eq. 1) was incorporated into the modified Luedeking-Piret model (Eq. 8) for $X(t)$, under conditions when S and t are equal to 0. The final product formation rate equation is shown in Eq. 9.

$$S(t) = S_0 - \gamma X_0 \left[\frac{e^{\mu_o t}}{1-\left(\frac{X_o}{X_m}\right)(1-e^{\mu_o t})} - 1 \right] - \delta \frac{X_m}{\mu_0} \ln[1 - (\frac{X_o}{X_m})(1 - e^{\mu_o t})] \quad (9)$$

Model validation

Model validation is important for showing the reliability of each model. In this study, the simulation models and the experimental data were evaluated using the linear correlation coefficient (R^2) and the root mean square deviation (RMSD). The R^2 value is frequently used to judge whether the model correctly represented the data; if R^2 is closer to 1, the regression model is correct. Meanwhile, the value of the RMSD is not standardized towards an acceptable range, but values close to zero are considered good. Teoh (2014) stated that a higher R^2 and the lower RMSD indicates a good fit.

Analytical Methods

Determination of biomass

The biomass was vacuum-filtered and oven-dried at 60 °C for 24 h before weighing. Biomass was then calculated using equation as shown in Eq. 10:

Biomass = Total biomass weight – initial weight of substrate (10)

Determination of glucose and ethanol concentration

Glucose in fermentation supernatants was analyzed by the dinitrosalicyclic acid (DNS) method, and the developed color was read on a spectrophotometer at 540 nm, as previously described (Ghose 1987). The ethanol concentration was determined by spectrophotometer readings at 267 nm (Magri *et al.* 1997).

RESULTS AND DISCUSSION

Bioethanol Production using Banana and Pineapple Wastes as Substrates

Figure 1(a) illustrates ethanol production during enzymatic hydrolysis experiments *via* SSCF. Banana and pineapple wastes inoculated with 10% (v/v) *A. terreus* and *K. marxianus* produced the maximum ethanol concentrations of 0.35 g/L and 0.27 g/L, respectively. Thus, cellular metabolism converted the reducing sugar to ethanol. Ethanol production was gradually increased throughout the fermentation period (Fig. 2). This result suggested that no mass transfer limitation was imposed in this experiment, which agreed with a previous conclusion that SSCF reduces end-product inhibition (Zhang *et al.* 2009). In terms of maximum productivity, banana wastes recorded the highest value at 0.07 g/L/d, which could be due to the ease of accessibility of the cells to reducing sugars compared with pineapple wastes with only productivity of 0.05 g/L/d.

Figure 1(b) represents the utilization of glucose during enzymatic hydrolysis *via* SSCF. All runs were almost identical, with a peak concentration at 12 h that decreased afterwards. Increases between 0 h and 12 h were due to the conversion of sucrose and enzymatic hydrolysis of cellulose into glucose and fructose by the invertase secreted by *A. terreus*. Zhang *et al.* (2009) supported this supposition by showing that SSCF involved

high sugar concentrations, particularly at the start of batch fermentation. The decreasing glucose concentration between 12 h and 120 h reflects its utilization by *K. marxianus*, either for biomass or ethanol production.

Selected Empirical Models of Fermentation Kinetics

The kinetic model described the relationship among the principal state variables and explained the behavior of fermentation quantitatively; hence, it provided useful information for the analysis, design, and operation of fermentation. Generally, the unstructured kinetic models were employed for modeling the microbial systems. These models are simple but sufficient for technical purposes (Teoh 2014).

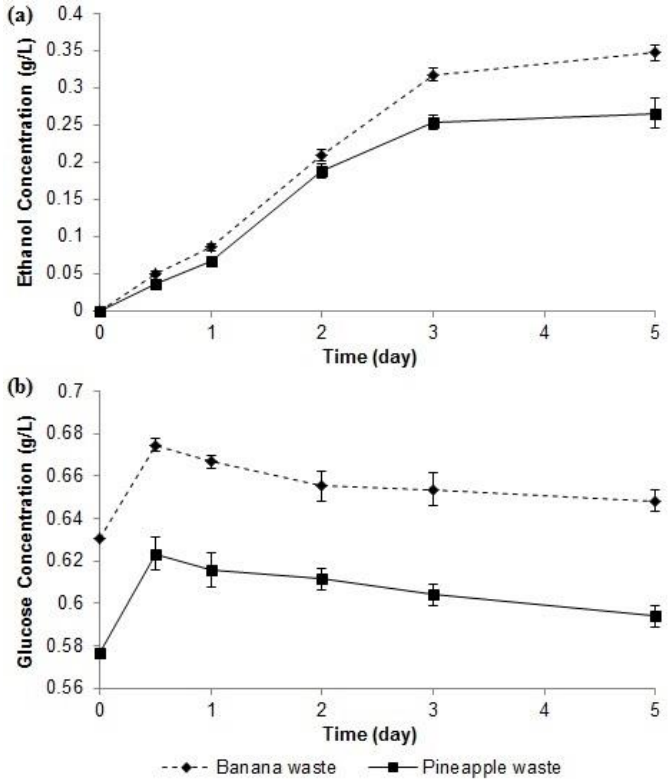

Fig. 1. (a) Ethanol concentration and (b) glucose concentration during batch SSCF

In this study, selected unstructured models were used to describe the kinetics of growth, product formation, and substrate consumption by *A. terreus* and *K. marxianus* in a batch fermentation system using banana and pineapple wastes as substrates. Each model was analyzed and validated based on the best-fit model, with the experimental data derived from the linear correlation coefficient (R^2) and the root mean square deviation (RMSD).

Microbial growth kinetics analysis

Table 1 summarizes the estimated parameters of the Logistic unstructured models for microbial growth profiles during SSCF. The results provided a good description with a regression coefficient of $R^2 > 0.9$. Figure 2 illustrates the microbial growth profile based on the experimental and calculated data. The Logistic model was the best adapted to the experimental data with the highest regression coefficient ($R^2 = 0.9816$) and the lowest

RMSD value for SSCF using banana waste. According to Baranyi (2010), the Logistic model produces a sigmoidal curve that represents both exponential and stationary phases.

Table 1. Estimated Parameters for Microbial Growth Based on the Logistic Model

Estimated Parameters	Substrate	
	Banana Waste	Pineapple Waste
X_o (g/L)	0.000006	0.0004
X_m (g/L)	20.3363	36.7419
μ_o (1/min)	0.0187	0.0037
R^2	0.9816	0.9283
RMSD	0.4909	1.6442

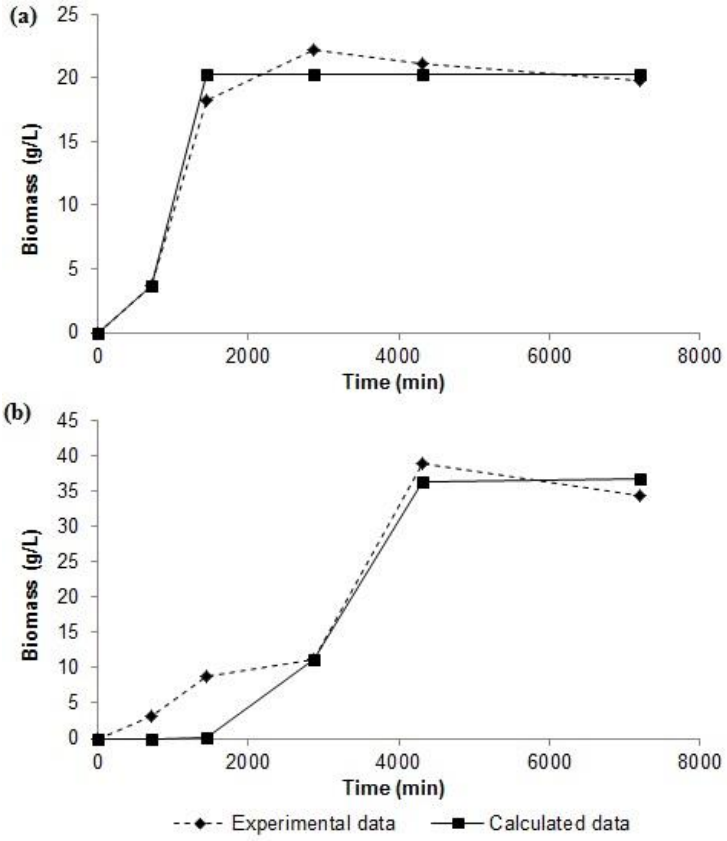

Fig. 2. Microbial growth using (a) banana waste and (b) pineapple waste, as determined experimentally or by the Logistic model

Product formation kinetic analysis

Bioethanol production was used as the main predictor of the kinetic parameters of product formation. The estimated kinetic parameters from the non-linear regression of the Logistic incorporated Luedeking-Piret models are listed in Eq. 5. Because the α and β values were not equal to zero (Table 2), the reaction followed a mixed-growth product associated formation. The experimental data for bioethanol production from banana and pineapple wastes fit well with the calculated data from these selected models, with R^2 equal to 0.98 and 0.91, respectively (Fig. 3).

Table 2. Estimated Parameters for Ethanol Production Based on the Logistic Incorporated Luedeking-Piret Model

Estimated Parameters	Substrate	
	Banana Waste	Pineapple Waste
P_o (g/L)	0.0051	0.00004
α (gP/gX)	0.4529	0.3661
β (gP/gX.min)	-0.00003	-0.0036
R^2	0.9888	0.9203
RMSD	0.0019	0.0178

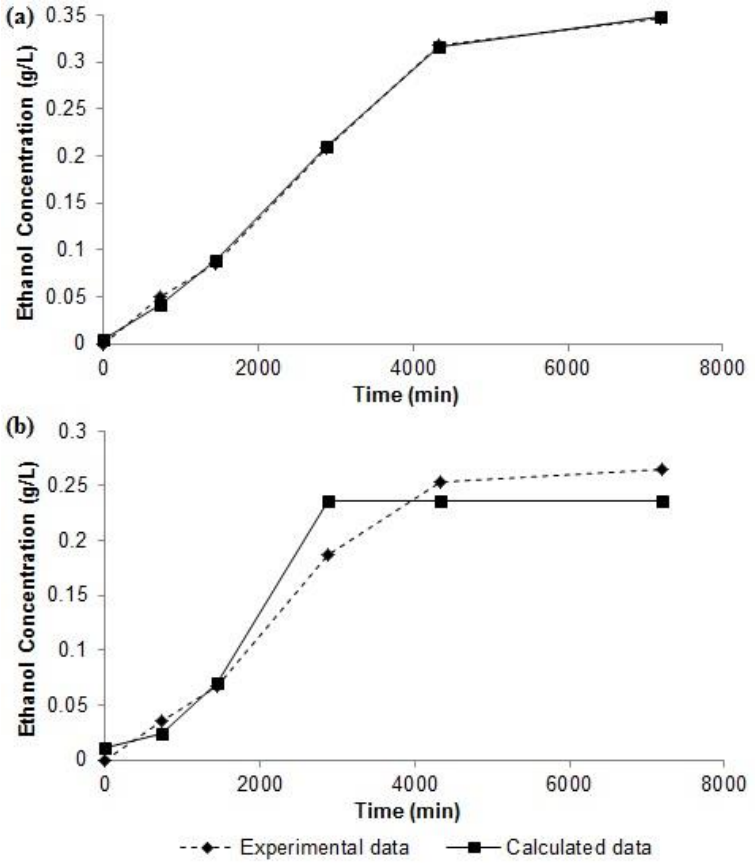

Fig. 3. Ethanol production during SSCF using (a) banana waste and (b) pineapple waste, as determined experimentally or by the Logistic incorporated Luedeking-Piret model

Substrate consumption kinetic analysis

Rajendran and Thangavelu (2008) stated that the consumption of a substrate was mainly for the growth rate, product formation, substrate consumption, and maintenance. The estimated kinetic parameters from the non-linear regression of the Logistic incorporated modified Luedeking-Piret models were tabulated in Table 3. As can be seen in Table 3 and Fig. 4, all experimental data fit well with the calculated data using the Logistic incorporated modified Luedeking-Piret model, with R^2 greater than 0.97 for both substrates tested.

Table 3. Estimated Parameters for Glucose Consumption Based on the Logistic Incorporated Modified Luedeking-Piret Model

Estimated Parameters	Substrate	
	Banana Waste	Pineapple Waste
S_o (g/L)	0.6304	0.5764
γ (gS/gX)	-0.2882	-29.3869
δ (gS/gX.min)	0.1745	0.0014
R^2	0.9762	0.9898
RMSD	0.0009	0.0006

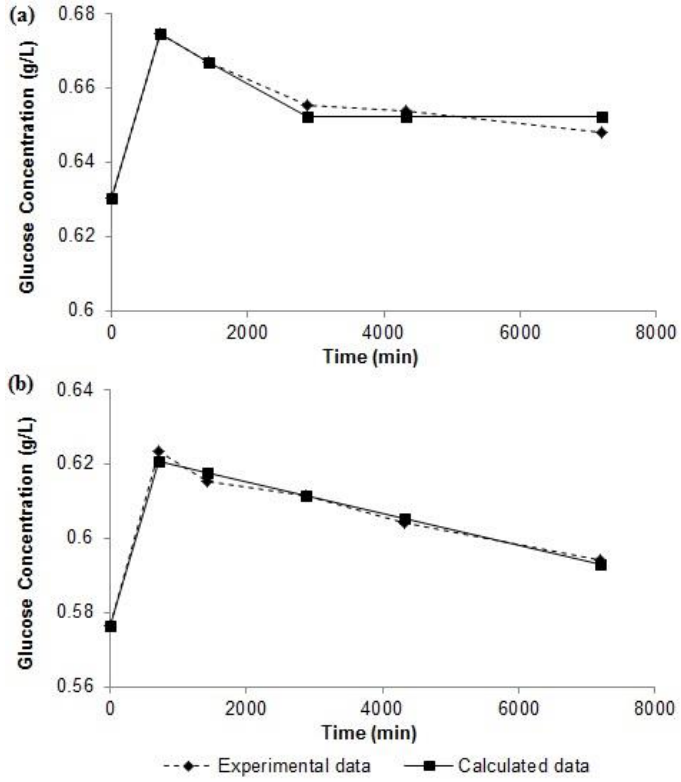

Fig. 4. Glucose consumption using (a) banana waste and (b) pineapple waste, as determined experimentally or by the Logistic incorporated modified Luedeking-Piret model

CONCLUSIONS

1. In this study, enzymatic hydrolysis using *Aspergillus terreus* and *Kluyveromyces marxianus* was performed *via* simultaneous saccharification and co-fermentation (SSCF) technology.
2. In terms of maximum productivity, the utilization of banana wastes recorded the highest value at 0.07 g/L/d.
3. Microorganism growth during batch fermentation was best described by the Logistic model, governing the lag, exponential, and stationary phase.

4. In addition, Logistic incorporated Luedeking-Piret models, as well as substrate utilization with a high R^2 value and a low RMSD, best described the production of bioethanol.

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